

**iGEM TU/e 2014**

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# Antifouling

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## 1 Stock solutions

- 300  $\mu\text{M}$  DBCO-PEG in DMSO: 250  $\mu\text{g}$  + 83.3  $\mu\text{L}$  DMSO
- 2.00  $\mu\text{M}$  Anti-HA antibody TAMRA
- Buffer: PBS with 0.1% BSA

## 2 Protocol

- Prepare following tubes: (6 in total)
- Make 3 of each tube type.

Tube	[DBCO]	Cells ( $10^7$ )	DBCO volume to add ( $\mu\text{L}$ ) (300 $\mu\text{M}$ )	DBCO/tag ratio
1	0	200 $\mu\text{L}$		
2	30 $\mu\text{M}$	200 $\mu\text{L}$	22.2	300.5

- Make sure you vortex the cell well before and after adding the DBCO-PEG
- React DBCO tubes for 1h to 6h in shaking block at 4°C and 300rpm
- Prepare FACS samples after 1h and 6h:
- Spin down the cells for 5 min at 13,400 rpm and discard the supernatant
- Resuspend with 1 mL ice cold PBS-0.1%BSA
- Spin down the cells for 5 min at 13,400 rpm and discard the supernatant
- Add 200  $\mu\text{L}$  ice cold PBS-0.1%BSA

## 3 Dilution of Antibodies

- Prepare following tubes: (Per DBCO 10kDa tube)

Tube	[Ab]	Antibody volume to add ( $\mu\text{L}$ ) 2.00 $\mu\text{M}$	DBCO?
1	-		
2	-		Yes
3	316 nM	33.82	
4	100 nM	10.19	
5	316 nM	33.82	Yes
6	100 nM	10.19	Yes

- Let the reaction happen for 1 hour
- Prepare FACS samples:
  - Spin down the cells for 5 min at 13,400 rpm
  - Discard the supernatant and resuspend with 1 mL ice cold PBS-0.1%BSA
  - Spin down the cells for 5 min at 13,400 rpm
  - Discard the supernatant and put the pellets on ice until FACS
  - Put on ice until FACS
  - Right before FACS: resuspend with 200  $\mu\text{L}$  ice cold PBS-0.1%BSA